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437-NF-03

PROCESS FOR PREPARING PYRROLO[2, 1-c] [1,4] BENZODIAZEPINE HYBRIDS

Field of the invention

The present invention relates to novel pyrrolo[2, 1-c] [1,4] benzodiazepine hybrids as well as processes for the preparation of novel pyrrolo[2, 1-c] [1,4] benzodiazepine hybrids. More particularly, present invention relates to a process for the preparation of novel pyrrolo[2, 1-c] [1,4] benzodiazepine hybrids as DNA sequence selective agents which are useful as potential antitumour agents. In particular, the present invention relates to a process for the preparation of new pyrrolo [2,1- c] [1,4] benzodiazepine hybrids as potential antitumour agents.

Background of the invention

Pyrrolo [2,1-c] [1,4] benzodiazepine antitumour antibiotics are commonly known as anthramycin class of compounds. In the last few years, a growing interest has been shown in the development of new pyrrolo [2,1-c] benzodiazepines (PBDs). These antibiotics react covalently with DNA to form an N2- guanine adduct that lies within the minor groove of duplex DNA via an acid-labile aminal bond to the electrophilic imine at the N10-C11 position (Kunimoto, S.; Masuda, T.; Kanbayashi, N. ; Hamada, M.; Naganawa, H.; Miyamoto, M.; Takeuchi, T.; and Unnezawa, H. J. Antibiot., 1980, 33, 665.; Kohn, K. w. and Speous, C. L. J. Mol., Biol., 1970, 51,551.; Hurley, L. H.; Gairpla, c. and Zmijewski, M. Biochem, Biophys. Acta., 1977, 475, 521,; Kaplan, D. J. and Hurley, L. H. biochmestry, 1981, 20, 7572). The molecules have a right – handed twist, which allows them to follow the curvature of the minor groove of B-form double-stranded DNA spanning three base pairs. Recently, PBD dimmers have been developed that comprises two C2- exo-methylene substituted DC-81 subunits tethered through their C-8 position I an inert propanedioxy linker (Gregson, S. J.; Howard, P. W; Hartely, J. A.; Brooks, N.a.; Adams, L.J.; Jenkins, T.C.; Kelland, L.R. and Thurston, D.E. J. Med Chem. 2001, 44, 737). A recent development has been the linking of two PBD units through their C-8 positions to give bisfunctional alkylating agents capable of cross- linking DNA (Thurston D. E.; Bose, D.S.; Thomson, a, S; Howard, P. W.; Leoni, A; Croker, S. J; Jenkins, T. C.; Neidle, S. and Hurley, L. H. J. Org. Chem., 1996, 61, 8141). Recently, a noncross linking mixed inmine-amide PBD dimmers have been synthesized that have significant DNA binding ability and potent anti tumour activity. (Kamal, A.; Ramesh, G.; Laxman, N; Ramulu, P,; Srinicas, O.; Neelima, K.;

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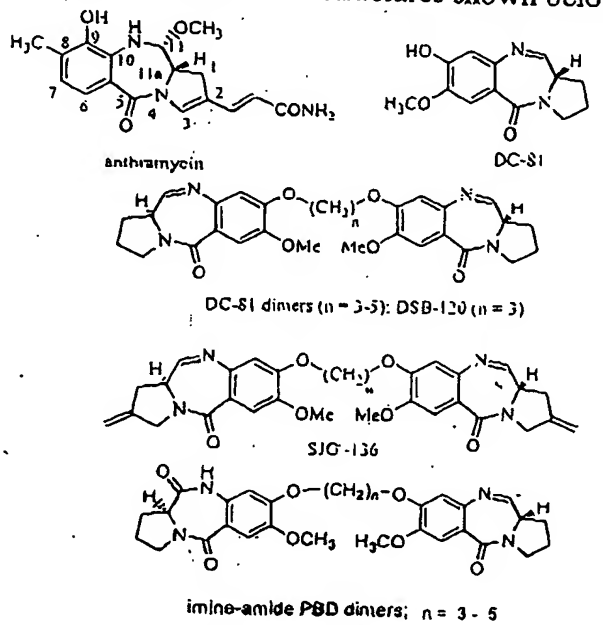
Kondapi, A. K.; Srinu, V. B,; Nagarajaram, H. M. J. Med. Chem. 2002, 45, 4679). These imine-amide PBD dimers have the structures shown below:



Naturally occurring pyrrolo [2, 1-c] [1,4] benzodiazepines belong to a group of antitumour antibiotics derived from Streptomyces Species. Recently, there is much impetus for the PBD system as they can recognize and bind to specific sequence of DNA. Examples of naturally occurring PBDs include anthramycin, DC-81, tomaymycin, sibiromycin and neothramycin.

However, the clinical efficacy for these antibiotics is hindered by several limitations, such as poor water solubility, cardiotoxicity, development of drug resistance and metabolic inactivation. There is therefore, a urgent need for such antibiotics which do have the disadvantages of the prior art.

Objects of the invention

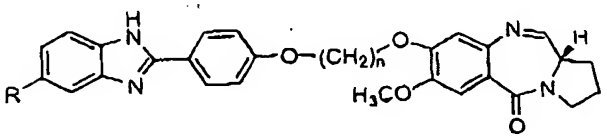
It is therefore am important object of the present invention is to provide a new pyrrolo [2, 1-c] [1, 4]- benzodiazepine hybrids useful as antitumour agents.

Another object of the present invention is to provide a process for the preparation of novel pyrrolo [2,1-c] 1,4]- benzodiazepine hybrids useful as antitumour agents.

Summary of the invention

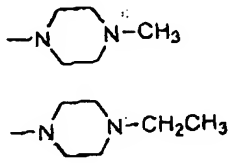
The above and othe objects of the present invention are achieved by providing a novel pyrrolo [2,1-c] [1,4] benzodiazepine hybrid compound. The present invention also provides a process for the preparation of 7-methoxy- 8-{n-[41H- benzo [d] imidazolo – 2 yl phenoxy] alkyl } – oxy (11aS) 1,2,3,-11 a tetrahydro- 5H- pyrrolo [

2,1- c] 1, 4] benzodiazepin- 5 one V, 7- methoxy- 8- (n –{ 4-[6- (4-methyl hexahydro-1- pyrainyl)- 1 H- benzo [d] imidazol- 2 yl] phenoxy } alkyl)- oxy- (11aS) – 1,2,3, 11a – tetrahydro – 5 H- pyrrolo 1 H- benzodiazepin 5 one V, 7- methoxy- 8 (n- {4- [6-4 methyl hexahydro-1 prazinyl)- 1H-benzo [d] imidazol- 2- yl [phenoxy) alkyl) oxy- (11aS)- 1,2,3, -11 a- tetrahydro-5H-pyrrolo [2,1-c) [1,4] benzodiazepin –5 one IX and 7- methoxy-8 (n- {4-[6-4-ethylhexahydro-1- pyraziny)- 1H- benzo [d] imidazol-2- yl] phenoxy) alkyl) – oxy- (11aS) – 1,2,3, -11a-tetra hydro 5H-pyrrolo [2,1-c) [1,4] bebzidiazepin- 5 one with aliphatic chain lenth variation of these compounds. These novel compounds also show DNA binding and anticancer (antitumour) activity. These novel pyrrolo [2,1,-c] [1,4] benzodiazepines have the general Formula XIV shown below:



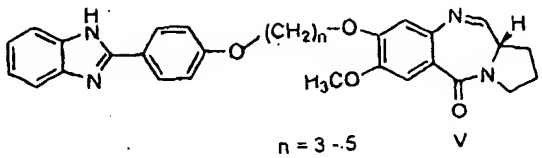
n = 3 - 5

R = H,

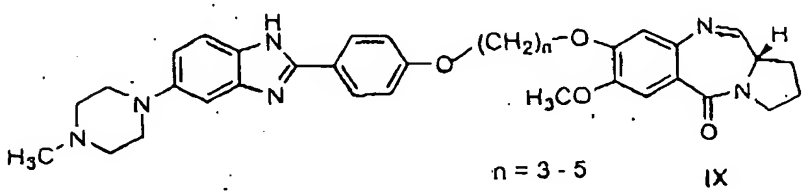


(XIV)

In a preferred embdodiment, present invention provides a process for the preparation of a novel pyrrolo [2,1-c] [1,4] benzodiazepine hybrids selected from the compounds of formulae V, IX and XIII wherein R= H, N- methylpypazine, N-ethylpyperazine and "n" is 3 to.5.



n = 3 - 5 V



n = 3 - 5 IX

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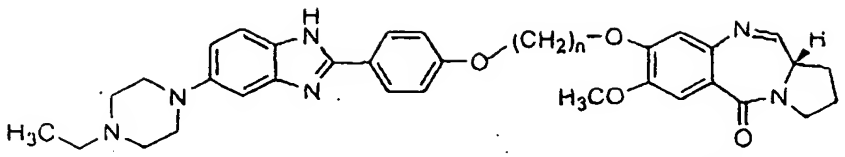
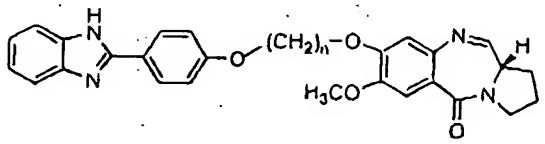


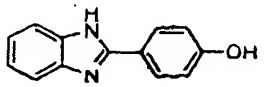
n = 3 - 5 XIII

Accordingly the present process provides a process for preparation of pyrrolo [2,1-c] 1, 4] benzodiazepine hybrids of formula V



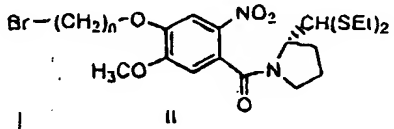
n = 3 - 5 V

which comprises reacting a 4- (1*H*- benzo[d] imidazol-2-yl) phenol of the formula I,



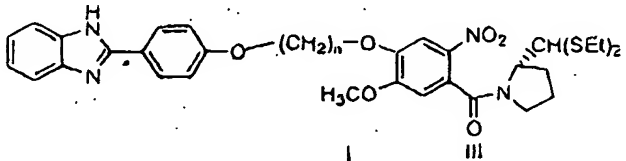
I

with N- [4-(n- bromoalkyloxy)-5- methoxyy-2- nitrobenzo-yl] pyrrolidine- 2-carboxaldehyde diethyl thio acetal of formula II



II

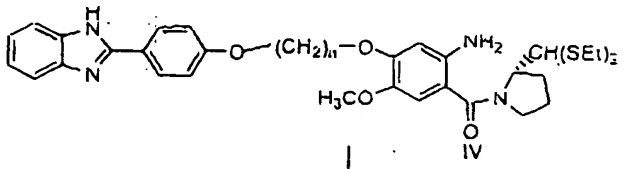
in the presence of K_2CO_3 in organic solvent for a period of 12 to 24 hrs, isolating (2S)-N- {4- (1*H*- benoz [d] imidazolo- 2 yl) phenoxy] alkyl – oxy- 5 methoxy- 2-nitrobenzoyl} pyrrolidine-2- carboxaldehyde diethyl thioacetal III



III

where "n" is 3 to 5, reducing said compound of formula III with $SnCl_2.2H_2O$ in the presence of organic solvent up to a reflux temperature, isolating the (2S) –N- {n- 4- (1

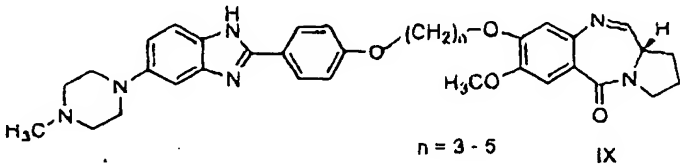
H- benzo [d] imidazolo- 2 yl) phenoxy] alkyl]-oxy- 5- methoxy- 2- aminobenzoyly) pyrrolidine- 2- carboxaldehyde diethyl thioacetal of the formula IV



IV

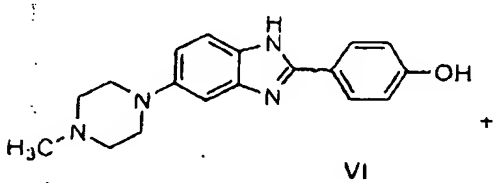
where n is 3 to 5 by known methods, reacting the said amino compound of formula IV with conventional deprotecting agents in to produce pyrrolo [2,1-c] 1, 4] benzodiazepine hybrids of formula V, wherein "n" is as defined above.

In another embodiment, the present invention provides a process for preparation of pyrrolo [2,1-c] 1, 4] benzodiazepine hybrids of formula IX



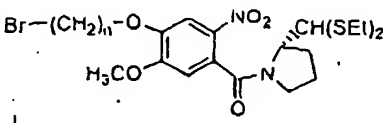
n = 3 - 5 IX

which comprises reacting a 4- [6-4- methylhexahydro- 1- pyrazinyl)- 1*H* – benzo[imidazol- 2- yl] phenol VI



VI

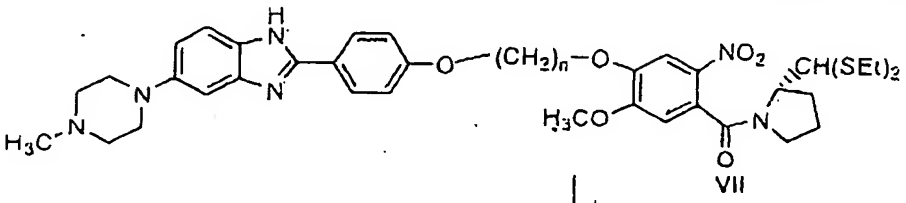
with N- [4-(n- bromoalkyloxy)-5- methoxyy-2- nitrobenzo-yl] pyrrolidine- 2- carboxaldehyde diethyl thio acetal of formula II



II

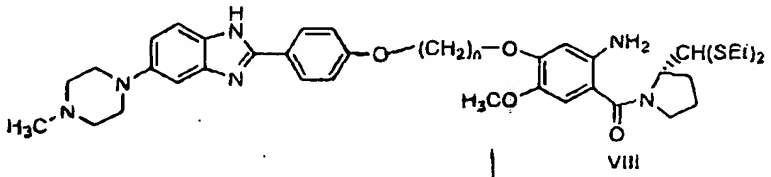
in the presence of K_2CO_3 in organic solvent for a period of 12 to 24 hrs, isolating (2S)- N- {n- (4- [6-4- methylhexahydro-1- pyraxinzyl)- 1H- benzo [d] imidazol- 2-yl]

phenoxy] alkyl-oxy- 5- methoxy-2- nitrobenzoy pyrrolidine-2- carboxaldehyde diethyl thioacetal VII



VII

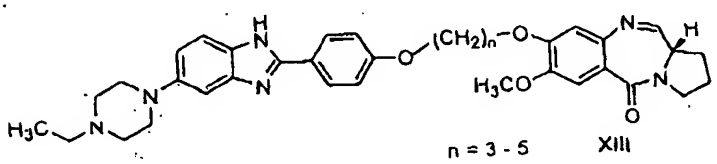
where "n" is 3 to 5, reducing said compound of formula VII with $SnCl_2.2H_2O$ in the presence of organic solvent up to a reflux temperature, isolating (2S)-N- {n- (4- [6-(4- methylhexahydro-1- pyrazinyl)- 1*H*- benzo [d] imidazol-2- yl] phenoxy] alkyl)-o xy- 5- methoxy –2- aminobenzoy} pyrrolidine-2- carboxaldehyde diethyl thioacetal VIII



VIII

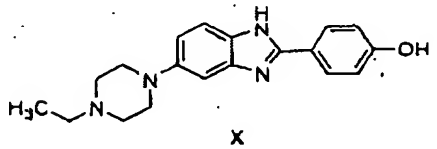
and reacting the said amino compound of formula VIII with conventional deprotecting agents in to produce pyrrolo [2,1-c] 1, 4] benzodiazepine hybrids of formula IX. wherein "n".

In yet another embodiment, the present invention provides a process for preparation of pyrrolo [2,1-c] 1, 4] benzodiazepine hybrids of formula XIII



n = 3 - 5 XIII

which comprises reacting a 4- [6-(4- ehtylhexahydro- I- pyrazinyl)- 1*H*- benzo [d] imidazol-2- yl] phenol X



X

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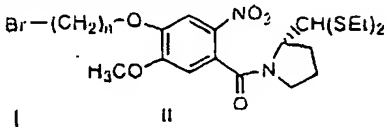
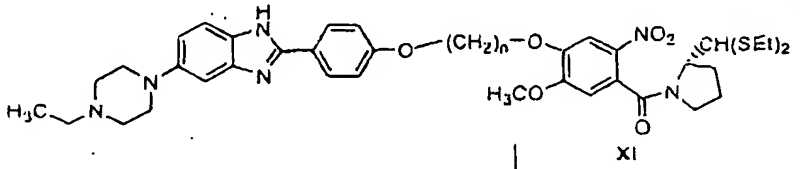
with N- [4-(n- bromoalkyloxy)-5- methoxyy-2- nitrobenzo-yl] pyrrolidine- 2-carboxaldehyde diethyl thio acetal of formula II



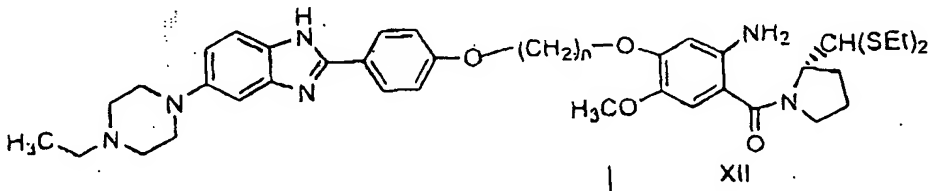
II

in the presence of K_2CO_3 in organic solvent for a period of 12 to 24 hrs, isolating (2S)-N- {n- (4- [6-4- ehtyhexahydro-1- pyrazinyl)- H- benzo [d] imidazol-2- yl] phenoxy] alkyl] – oxy- 5- methoxy- 2- nitrobenzoyl} pyrrolidine- 2- carboxaldehyde diethyl thioacetal XI



XI

where "n" is 3 to 5, reducing said compound of formula XI with $SnCl_2.2H_2O$ in the presence of organic solvent up to a reflux temperature, isolating (2S)-N- {n-(4-[6-(4-ehtylhexahydro-1- pyrazinyl)-1*H* – benzo[d] imidazol-2- yl] phenoxy] alkyl)- oxy-5-methoxy-2- aminobenzoyl} pyrrolidine- 2- carboxaldehyde diethyl thioacetal XII where n is 3 to 5



XII

and reacting the said amino compound of formula XII with conventional deprotecting agents to produce pyrrolo [2,1-c] 1, 4] benzodiazepine hybrids of formula XIII wherein "n" is as defined above..

Detailed Description

The precursors, 4- (1H- benzo [d] imidazo 1-2-yl) phenol 1, 4- [6- (4-methylhexadhyro-1- pyrazinyl)- 1H- benzo [d] imidazo 1-2- yl] phenol VI and 4- [6-(4 ehtylhexahydro-1- pyrazinyl)- 1H- benzo[d] dimidazol-2-yl] phenol X (Ji, Yu,; Hasler , W,; Schmitt, V. R.; Dorn, A.; Baily C.; Waring, M.J.; Hochstrasser, R.; Keupin, W. Bioorg Med Chem Lett. 2001, 9, 2905) and (2S)-N- [4-(b- bromoalkyloxy)- 5-methoxy- 2- nitrobenzo- yl] pyrrolidine-2-carboxaldehyde diethyl thioacetal II (Kamal,

A.; Ramesh, G.; Laxman, N.; Ramulu, P.; Srinivas, O,; Neelima, K.; Kondapi, A. K.; Srinu, V. B.; Nagarajaram H. M. J. Med. Chem. 2002, 45,4679) have been prepared by literature methods.

Some representative compound of formulae V/IX/XIII of present invention are given below

1. 7-Methoxy –8- n{3-[4- (1 H- benzo [d] imidazolo- 2 yl) phenoxyl] propoxy} – (11aS) 1,2,3- 11a – tetrahydro- 5H – pyrrolo [2,1-c] 01,4] benzodiazepin- 5- one. 1
2. 7-Methoxy –8- {4- [4-)1H-benzo [d] imidazolo- 2yl phenoxy] butoxy} – (11aS) 1,2,3-11a-tetrahydro- 5H- pyrrolo[2,1- c] [1,4] benzodiazepin –5- one
3. 7- Methoxy- 8- {5 – [4-(1H- benzo [d] imidazolo – 2yl) phenoxyl] pentyloxy} – (11aS) 1,2,3,-11a-tetrahydro-5H- pyrrolo {2, 1-c] [1, 4] benzodiazepin-5- one
4. 7- methoxy –8- (3-{4-[6-(4- methylhexahydro- 1- pyrazinyl)- 1H- benzol [d] imidazol-2- yl] phexnoxy} propoxy) – (11aS)- 1,2,3-11 a- tetrahydro- 5 H- pyrrolo [2,1-c]
5. 7-methoxy-8- (4-{4-[6-(4- methylhexaydro-1- pyrazinyl)-1H- benzol [d] imidazol-2- yl] phenoxy} butoxy)- (11aS) –1,2,3,-11 a- tetrahydro-5 H- pyrrolo [2,1-c] [1,4] benzodiazepin- 5- one
6. 7- methoxy- 8- (5- {4-[6- (4- methylhexahydro- 1 – pyrazinyl)-1 H- benzo[d] imidazol-2- yl] phenoxy} pentyloxy)- (11aS)- 1,2,3- 11a- tetrahydro-5- pyrrolo [2,1-c)
   [1,4] benzodiazepin –5- one
7. 7-methoxy-8- (3-{4-[6-(4- ethyexahydro-1- pyrazinyl)-1H- benzo[d] imidazol-2- yl] phenoxy)- (11aS)- 1,2,3,-11 a- tetrahydro- 5H- pyrrolo [2, 1- c]
   [1,4]benzodiazepin- 5 - one
8. 7- methoxy-8- (4-{4-[6-(4- ethylhexahydro-1- pyrazinyl)- 1 H- benzo [d] imidazol-2- yl] phenoxy } butoxy- (11aS)- 1,2,3-11a- tetrahydro- 5H- pyrrolo [2,1-c]
   [1,4] benzodiazepin –5- one
9. 7- methoxy-8- (5-{4-[6-(4- ethylhexahydro-1- pyrazinyl)- 1 H- benzo [d] imidazol-2- yl] phenoxy } pentyloxy- (11aS)- 1,2,3-11a- tetrahydro- 5H- pyrrolo [2,1-c]
   [1,4] benzodiazepin –5- one

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These new analogues of pyrrolo [2,1-c] [1,4] benzodiazepin hybrids linked at C-8 position have shown promising DNA binding activity in various cell lines. The molecules synthesized are of immense biological significance with potential sequence selective DNA – binding property. This resulted in design and synthesis of new congeners as illustrated in the following reaction Schemes which comprise:

1. The either linkage at C-8 position of DC-81 intermediates with substituted 2-phenoxy benzimadazole moiety.
2. Up to refluxing the reaction mixture for 12-48 h.
3. Synthesis of C-8 linked PBD antitumour antibiotic hybrid imines.
4. Purification by column chromatography using different solvents like ethyl acetate hexane, dichloromethane, chlorform and methanol.

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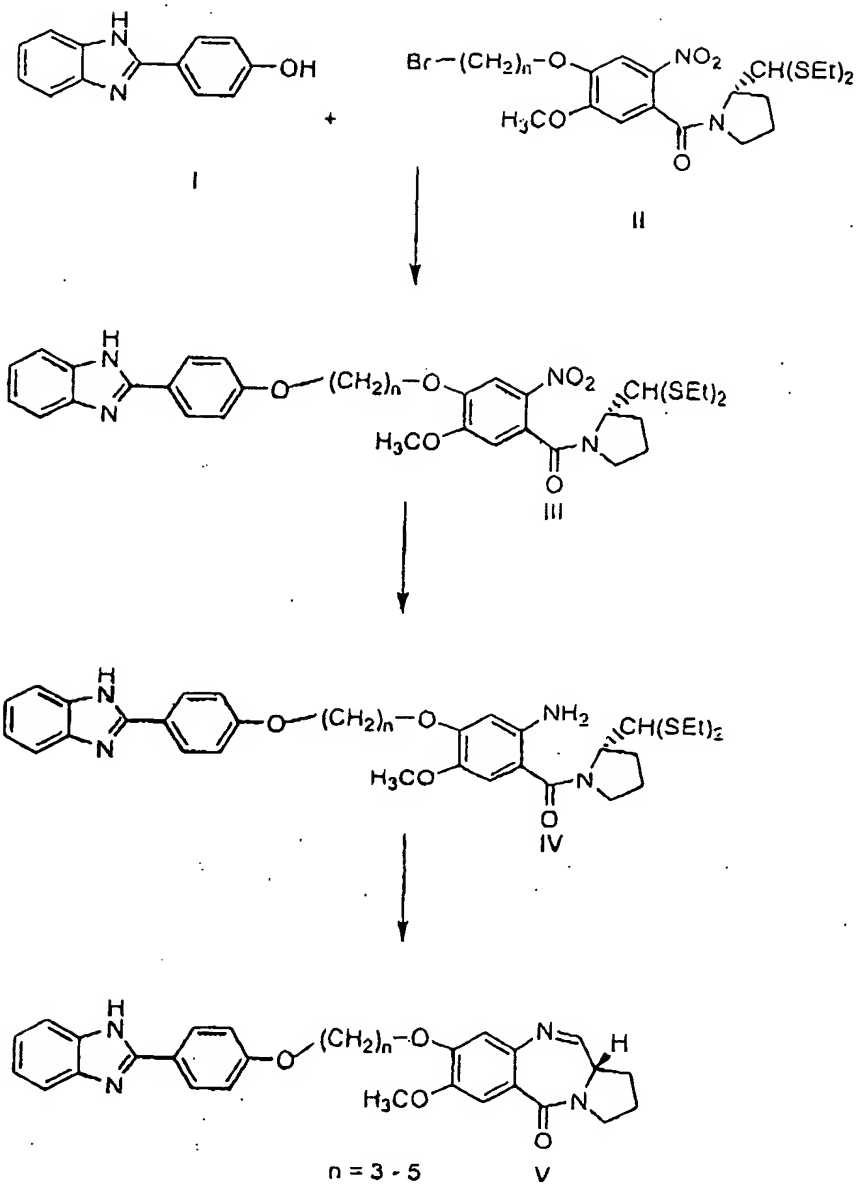
Reaction Schemes



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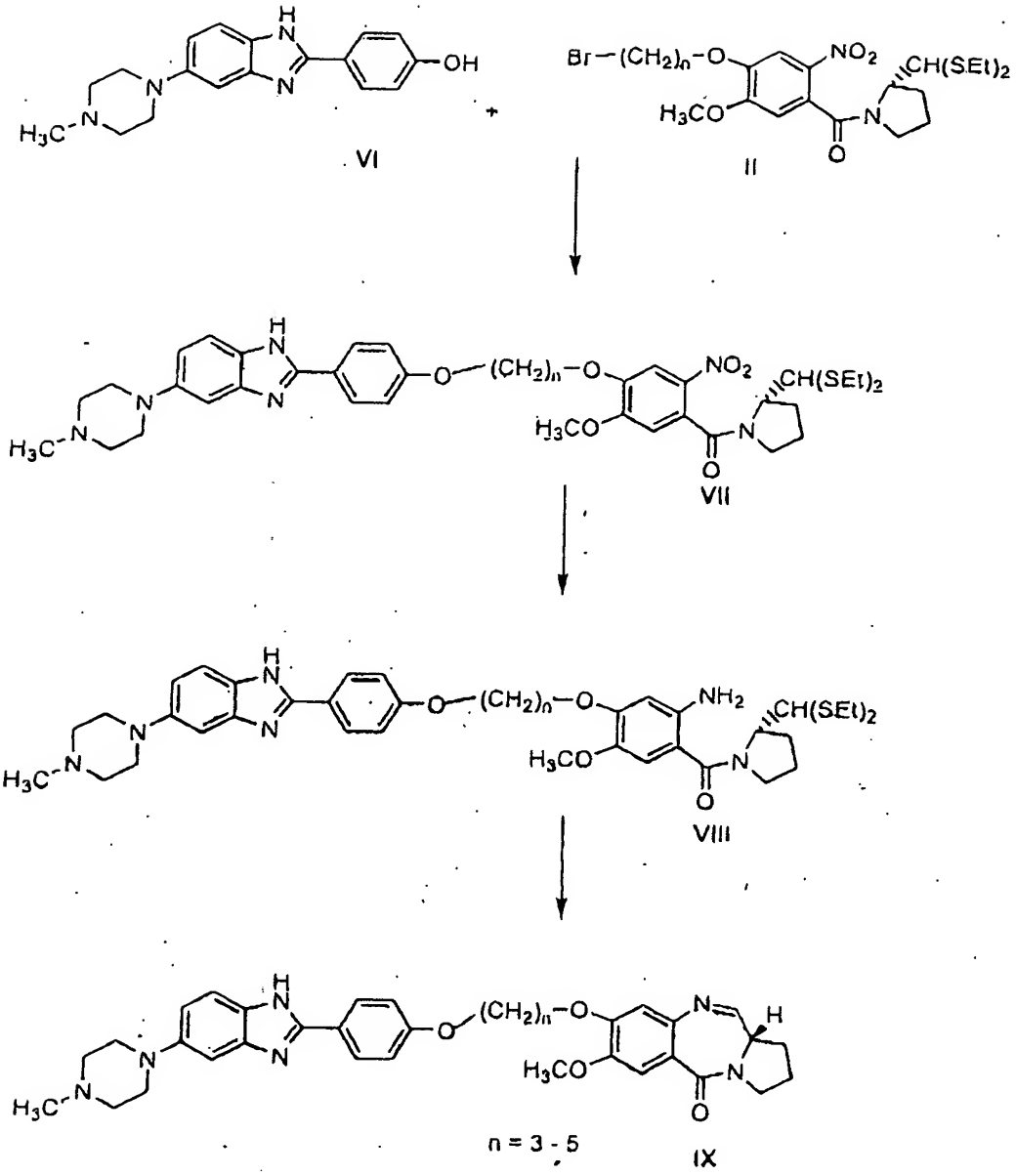

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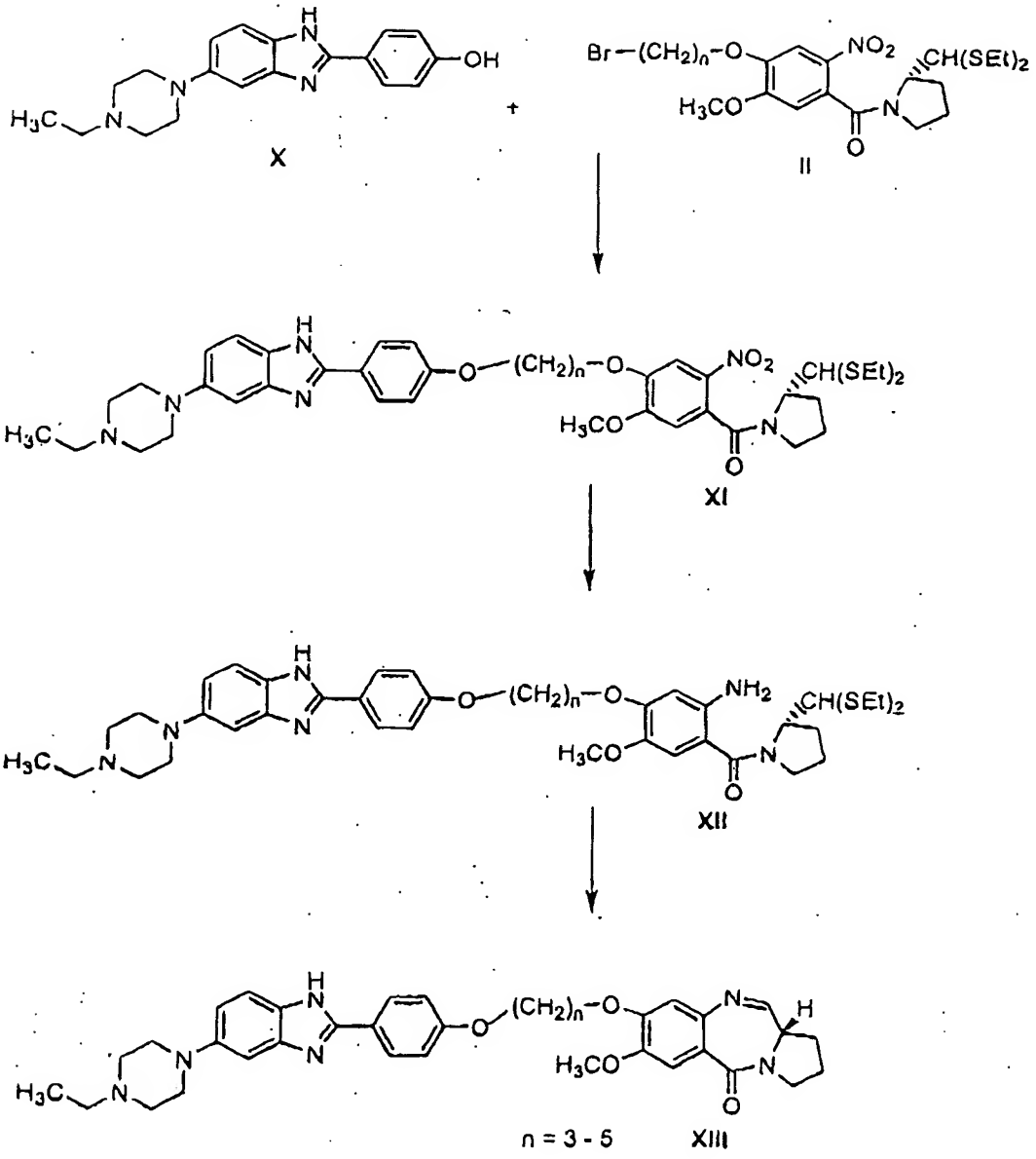

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The invention will now be described with reference to the following example, which are given by way of illustration and therefore should not be construed to the present limit of the scope of invention.

Example 1

Compound 4-[1H-benzo [d] imidazol-2-yl] phenol I (210 mg, 1 mmol) and (2S)-N-4-(3-bromobutyloxy)-5-methoxy-2-nitrobenzoyl] pyrrolidin-2-carboxaldehyde diethyl thioacetal of formula II (521 mg, 1 mmol) was taken in dry DMF (10mL). K2CO3 (690mg, 5mmol) was added and the mixture was stirred for 12 to 24hrs. The reaction mixture was poured in to ice-water then solid was formed and it was filtered and aqueous media was extracted with EtOAc and $CHCl_3$ (50 mL), then the extracted solution was evaporated in vacuum to obtain the solid compound. Two solids were combined and the crude material was. chromatographed over silica gel using chloroform / methanol (8:2) solvent to give compound (2S)–N-{3-(4-(1*H*-benzo[d] imidazol-2-yl] phenoxy) propoxy-5-method-2-nitrobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal VII as a sticky solid.

The compound (2S)–N-{3-(4-(1*H*-benzo[d] imidazol-2-yl] phenoxy) propoxy-5-method-2-nitrobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal III (0.649 mg, 1 mmol) was dissolved in methanol (15 ml) and added $SnCl_2.2H_2O$ (1.13 g, 5 mmol) was refluxed fro 2-5 h or until the TLC indicated that reaction was completed. The reaction mixture was then adjusted to pH 8 carefully with saturated $NaHCO_3$ solution, diluted with ethyl acetate, filtered through celite and extracted. The combined organic phase was dried over Na_2SO_4, and evaporated under vacuum to afford the crude compound (2S)–N-{3-(4-(1*H*-benzo[d] imidazolo-2-yl] phenoxy) propoxy-5-method-2-aminobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal VIII.

A solution of compound (2S)–N-{3-(4-(1*H*-benzo[d] imidazolo-2-yl] phenoxy) propoxy-5-method-2-aminobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal IV (619 mg, 1 mmol), $HgCl_2$ (613 mg, 2.26 mmol) and CaCO3 (246 mg, 2.46 mmol) in MeCN-water (4:1) was stirred slowly at room temperature until TLC indicates completed loss of starting material. The reaction mixture was diluted with EtOAc (30mL) and filtered through a celite bed. The clear brown organic supernatant was extracted with saturated 5% $NaHCO_3$ (20mL), brine (20mL) and the combined organic phase was dried ($Na2SO_4$). The organic layer was evaporated in vacuum and purified by column chromatography (95% CH_2Cl_2-MeOH) to give compound 7-methoxy-8-(3-

[4-[1H-benzo[d] imidazol-2-yl] phenoxy} protoxy)-(11aS)-1, 2, 3, - 11a-tetrahydro-5*H*-pyrrolo[2,1-c] [1,4] benzodiazepin-5-one.

^{1}H NMR ($CDCl_3$) & 1.90-2.10 (m,2H), 2.20-2.39 (m, 4H), 3.90 (s, 3H), 3.90 (m,3H), 4.10-4.30 (m, 4H), 6.80-6.98 (3s,3H), 7.10-7.30 (m, 2H), 7.45 (s,1H), 7.5-7.65 (m, 3H), 7.85-7-.90 (d, 2H); MS (FAB) 497 $[M+H]^+$.

Example 2

Compound 4-[1*H*-benzo [d] imidazol-2-yl] phenol I (210 mg, 5 mmol) and (2S)-N-[4-(4-bromobutyloxy)-5-methoxy-2-nitrobenzoyl] pyrrolidin-2-carboxaldehyde diethyl thioacetal of formula II (535 mg, 1 mmol) was taken in dry DMF (10mL). K_2CO_3 (690 mg, 5 mmol) was added and the mixture was stirred for 12 to 24hrs. The reaction mixture was poured in to ice-water then solid was formed and it was filtered and aqueous media was extracted with EtOAc and $CHCl_3$ (50 mL). Then the extracted solution was evaporated in vacuum to obtain the solid compound. Two solids were combined and the crude material was chromatographed over silica gel using chloroform / methanol (8:2) solvent to give compound (2S)–N-{3-(4-(1*H*-benzo[d] imidazol-2-yl] phenoxy) propoxy] butoxy-5-method-2-nitrobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal III as a sticky solid.

The compound (2S)–N-{4-(4-(1*H*-benzo[d] imidazol-2-yl] phenoxy)-5-methoxy-2-nitrobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal III (633 mg, 1 mmol) was dissolved in methanol (15 ml) and added with $SnCl_2.2H_2O$ (1.12 g, 5 mmol) and was refluxed for 2-5 h or until the TLC indicated that reaction was completed. The reaction mixture was then adjusted to pH 8 carefully with saturated $NaHCO_3$ solution, diluted with ethyl acetate, filtered through celite and extracted. The combined organic phase was dried over Na_2SO_4, and evaporated under vacuum to afford the crude compound (2S)–N-{3-(4-(1*H*-benzo[d] imidazolo-2-yl]phenoxy] butoxy-5-methoxy -2-aminobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal IV.

A solution of compound (2S)–N-{4-(4-(1*H*-benzo[d] imidazolo-2-yl] phenoxy) butoxy-5-method-2-aminobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal IV (603 mg, 1 mmol), $HgCl_2$ (613 mg, 2.26 mmol) and $CaCO_3$ (246 mg, 2.46 mmol) in MeCN-water (4:1) was stirred slowly at room temperature until TLC indicates completed loss of starting material. The reaction mixture was diluted with EtOAc (30mL) and filtered through a celite bed. The clear brown organic supernatant was extracted with saturated 5% $NaHCO_3$ (20mL), brine (20mL) and the combined organic phase was dried (Na_2SO_4). The organic layer was evaporated in vacuum and purified by

column chromatography (95% CH_2Cl_2-MeOH) to give compound 7-methoxy-8-(3-[4-[1H-benzo[d] imidazol-2-yl] phenoxy} butoxy)-(11aS)-1,2,3,-11a-tetrahydro-5*H*-pyrrolo[2,1-c] [1,4] benzodiazepin-5-one.

^1H NMR ($CDCl_3$) & 1.80-2.20 (m,6H), 2.21-2.42 (m, 2H), 3.50-3.95 (m, 6H), 4.05-4.30 (m,4H), 6.70-682 (m, 3H), 7.19-7.21 (m, 2H), 7.3 (s, 1H), 7.59-7.70 (m, 3H), 7.80-7.70 (m, 3H), 7.80-7.90 (d, 2H); MS (FAB) 511$[M+H]^+$.

Example 3

Compound 4-[1*H*-benzo [d] imidazol-2-yl] phenol I (210 mg, 1 mmol) and (2S)-N-[4-(5-bromobutyloxy)-5-methoxy-2-nitrobenzoyl] pyrrolidin-2-carboxaldehyde diethyl thioacetal of formula II (549 mg, 5 mmol) was taken in dry DMF (10mL), K_2CO_3 (690 mg, 5 mmol) was added and the mixture was stirred for 12-24th. The reaction mixture was poured in to ice-water then solid was formed and it was filtered and aqueous media was extracted with EtOAc and $CHCl_3$ (50 mL). Then the extracted solution was evaporated in vacuum to obtain the solid compound. Two solids were combined and the crude material was. chromatographed over silica gel using chloroform / methanol (9:1) solvent to give compound (2S)–N-{5-(4-(1*H*-benzo-[d] imidazol-2-yl] phenoxy) pentyloxy-5-method-2-nitrobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal III as a sticky solid.

The compound (2S)–N-{5-(4-(1*H*-benzo[d] imidazol-2-yl] phenoxy) pentyloxy-5-methoxy-2-nitrobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal III (0.647 g, 1 mmol) was dissolved in methanol (15 ml) and added $SnCl_2.2H_2O$ (1.12 g, 5 mmol) was refluxed fro 2-5 h or until the TLC indicated that reaction was completed. The reaction mixture was then adjusted to pH 8 carefully with saturated $NaHCO_3$ solution, diluted with ethyl acetate, filtered through celite and extracted. The combined organic phase was dried over Na_2SO_4, and evaporated under vacuum to afford the crude compound (2S)–N-{5-(4-(1*H*-benzo[d] imidazolo-2-yl]phenoxy] pentyloxy-5-methoxy -2-aminobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal IV.

A solution of compound (2S)–N-{5-(4-(1*H*-benzo[d] imidazolo-2-yl] phenoxy) pentyloxy-5-method-2-aminobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal III (617 mg, 1 mmol), $HgCl_2$ (613 mg, 2.26 mmol) and $CaCO_3$ (246 mg, 2.46 mmol) in MeCN-water (4:1) was stirred slowly at room temperature until TLC indicates completed loss of starting material. The reaction mixture was diluted with EtOAc (30mL) and filtered through a celite bed. The clear brown organic supernatant was extracted with saturated 5% $NaHCO_3$ (20mL), brine (20mL) and the combined organic

phase was dried (Na_2SO_4). The organic layer was evaporated in vacuum and purified by column chromatography (95% CH_2Cl_2-MeOH) to give compound 7-methoxy-8-(5-[4-[1H-benzo[d] imidazol-2-yl] phenoxy} pentyloxy)-(11aS)-1,2,3,-11a-tetrahydro-5*H*-pyrrolo[2,1-c] [1,4] benzodiazepin-5-one.

^1H NMR ($CDCl_3$) & 1.60-2.19 (m, 8H), 2.25-2.39 (m, 2H), 3.60-4.20 (m, 10H), 6.70-6.90 (m,3H), 7.19-7.30 (m, 2H), 7.50 (s, 1H), 7-.65-7.78 (m, 3H), 7.90-8.01 (d, 2H); MS (FAB) 525$[M+H]^+$.

Example 4

Compound 4-[6-(4-methylhexahydro-1-pyrazinyl)-1*H*-benzo [d] imidazol-2-yl] phenol VI (328 mg, 1 mmol) and (2S)-N-[4-(3-bromobutyloxy)-5-methoxy-2-nitrobenzoyl] pyrrolidin-2-carboxaldehyde diethyl thioacetal of formula II (521 mg, 1 mmol) was taken in dry DMF (10mL). K_2CO_3 (690 mg, 5 mmol) was added and the mixture was stirred for 12-24th. The reaction mixture was poured in to ice-water then solid was formed and it was filtered and aqueous media was extracted with EtOAc and $CHCl_3$ (50 mL), then the extracted solution was evaporated in vacuum to obtain the solid compound. Two solids were combined and the crude material was. chromatographed over silica gel using chloroform / methanol (9:1) solvent to give compound (2S)–N-{3-(4-(6(4-Methylhexahydro-1-pyrazinyl)-1H-benzo [d] imidazol-2-yl] phenoxy) propoxy-5-methoxy-2-nitrobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal VII as a stick solid.

The compound (2S) –N-{3-(4-[6-(4-methylhexahydro-1-pyrazinyl)-1H-benzo [d] imidazol-2-yl] phenoxy) propoxy-5-methoxy-2-nitrobenzoyll} pyrrolidine-2-carboxaldehyde diethyl thioacetal VII (0.767 g, 1 mmol) was dissolved in methanol (15 ml) and added $SnCl_2.2H_2O$ (1.12 g, 5 mmol) was refluxed fro 5-7 h or until the TLC indicated that reaction was completed. The reaction mixture was then adjusted to pH 8 carefully with saturated $NaHCO_3$ solution, diluted with ethyl acetate, filtered through celite and extracted. The combined organic phase was dried over Na_2SO_4, and evaporated under vacuum to afford the crude compound (2S) –N-{3-(4-[6-(4-methylhexahydro-1-pyrazinyl)-1H-benzo[d]imidazol-2-yl] imidazol-2-yl]phenoxy)propoxy-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal VIII.

A solution of compound (2S) –N-{3-(4-[6-(4-methylhexahydro-1-pyrazinyl)-1H-benzo[d]imidazol-2-yl] imidazol-2-yl]phenoxy)propoxy-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal VIII (637 mg, 1 mmol),

$HgCl_2$ (613 mg, 2.26 mmol) and CaCO3 (246 mg, 2.46 mmol) in MeCN-water (4:1) was stirred slowly at room temperature until TLC indicates completed loss of starting material. The reaction mixture was diluted with EtOAc (30mL) and filtered through a celite bed. The clear brown organic supernatant was extracted with saturated 5% $NaHCO_3$ (20mL), brine (20mL) and the combined organic phase was dried (Na2SO4). The organic layer was evaporated in vacuum and purified by column chromatography (80% CH2Cl2-MeOH) to give compound 7-methoxy-8-(3-[4-[6-(4-methylhexahydro-1-pyraziny)-1H-benzo[d] imidazol-2-y1] phenoxy} propoxy)-(11aS)-1, 2, 3, - 11a-tetrahydro-5H-pyrrolo[2,1-c] [1,4] benzodiazepin-5-one.

1H NMR ($CDCl_3$) & 1.90-2.15 (m,3H), 2.20-2.30 (m, 3H), 2.40 (s, 3H), 2.60-2.75 (m,4H), 3.10-3.20 (m, 3H), 3.90 (s,3H), 4.10-4.35 (m, 4H), 6.75 (s, 1H), 6.80-7.1 (m, 5H), 7.60-7.70 (d, 1H, J=4.4 Hz), 7.90-8.10 (d, 2H): MS (FAB) 595 $[M+H]^+$.

Example 5

Compound 4-[6-(4-methylhexahydro-1-pyrazonyl)-1H-zenzo [d] imidazol-2-yl] phenol VI (328 g, 1 mmol) and (2S)-N-4-(4-bromobutyloxy)-5-methoxy-2-nitrobenzoy1] pyrrolidin-2-carboxaldehyde diethyl thioacetal of formula II (535 mg, 1 mmol) was stirred for 12-24th. The reaction mixture was poured in to ice-water then solid was formed and it was filtered and aqueous media was extracted with EtOAc and CHCl3 (50 mL), then the extracted solution was evaporated in vacuum to obtain the solid compound. Two solids were combined and the crude material was. Chromatographed over silica gel using chloroform / methanol (8:2) solvent to give compound (2S)–N-{4-(4-[6-(4-methylhexahydro-1-pyrazinyl)-1*H*-benzo[d] imidazol-2-yl] phenoxy) butoxy-5-method-2-nitrobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal VII as a sticky solid.

The compound (2S) –N-{4-(4-[6-(4-methylhexahydro-1-pyrazinyl)-1*H*-benzo [d] imidazol-2-yl] phenoxy) propoxy-5-methoxy-2-nitrobenzoyll} pyrrolidine-2-carboxaldehyde diethyl thioacetal VII (0.781 g, 1 mmol) was dissolved in methanol (15 ml) and added $SnCl_2.2H_2O$ (1.12 g, 5 mmol) was refluxed fro 5-7 h or until the TLC indicated that reaction was completed. The reaction mixture was then adjusted to pH 8 carefully with saturated $NaHCO_3$ solution, diluted with ethyl acetate, filtered through celite and extracted. The combined organic phase was dried over Na_2SO_4, and evaporated under vacuum to afford the crude compound (2S) –N-{4-(4-[6-(4-methylhexahydro-1-pyrazinyl)-1H-benzo[d]imidazol-2-yl] imidazol-2-

yl]phenoxy)propoxy-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal VIII.

A solution of compound (2S) –N-{4-(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo[d]imidazol-2-yl] imidazol-2-yl]phenoxy)propoxy-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal VIII (751 mg, 1 mmol), $HgCl_2$ (613-mg, 2.26 mmol) and CaCO3 (246 mg, 2.46 mmol) in MeCN-water (4:1) was stirred slowly at room temperature until TLC indicates completed loss of starting material. The reaction mixture was diluted with EtOAc (30mL) and filtered through a celite bed. The clear brown organic supernatant was extracted with saturated 5% $NaHCO_3$ (20mL), brine (20mL) and the combined organic phase was dried ($Na2SO_4$). The organic layer was evaporated in vacuum and purified by column chromatography (80% CH_2Cl_2-MeOH) to give compound 7-methoxy-8-(4-[4-[6-(4-methylhexahydro-1-pyraziny)-1H-benzo[d] imidazol-2-yl] phenoxy} butoxy)-(11aS)-1, 2, 3, - 11a-tetrahydro-5H-pyrrolo[2,1-c] [1,4] benzodiazepin-5-one.

1H NMR ($CDCl_3$) & 1.80-2.18 (m,3H), 2.35 (m, 3H), 2.60-2.70 (s, 4H), 3.10-3.20 (m,4H), 3.60-3.80 (m, 3H), 3.90 (s,3H), 4.01-4.25 (m, 4H), 6.72 (s, 1H), 7.35 (m, 5H), 7.61-7.30 (d, 1H, J=3.6 Hz), 7.98-8.03 (d, 2H): MS (FAB) 6.9 $[M+H]^+$.

Example 6

Compound 4-[6-(4-methylhexahydro-1-pyrazonyl)-1*H*-zenzo [d] imidazol-2-yl] phenol VI (328 g, 5 mmol) and (2S)-N-4-(5-bromobutyloxy)-5-methoxy-2-nitrobenzoyl] pyrrolidin-2-carboxaldehyde diethyl thioacetal of formula II (549 mg, I mmol) was taken in dry DMF (10mL). K2CO3 (690mg, 5 mmol) was added and the mixture was stirred for 12-24th. The reaction mixture was poured in to ice-water then solid was formed and it was filtered and aqueous media was extracted with EtOAc and CHCl3 (50 mL), then the extracted solution was evaporated in vacuum to obtain the solid compound. Two solids were combined and the crude material was. Chromatographed over solica gel using chloroform / methanol (8:2) solvent to give compound (2S) –N-{5-(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo[d] imidazol-2-yl] phenoxy) pentyloxy-5-method-2-nitrobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal VII as a sticky solid.

The compound (2S) –N-{5-(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo [d] imidazol-2-yl] phenoxy) propoxy-5-methoxy-2-nitrobenzoyll} pyrrolidine-2-carboxaldehyde diethyl thioacetal VII (0.795 g, 1 mmol) was dissolved in methanol (15 ml) and added $SnCl_2.2H_2O$ (1.12 g, 5 mmol) was refluxed fro 5-7 h or until the TLC

indicated that reaction was completed. The reaction mixture was then adjusted to pH 8 carefully with saturated $NaHCO_3$ solution, diluted with ethyl acetate, filtered through celite and extracted. The combined organic phase was dried over Na_2SO_4, and evaporated under vacuum to afford the crude compound (2S) –N-{5-(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo[d]imidazol-2-yl] imidazol-2-y1]phenoxy) pentyloxy -5-methoxy-2-aminobenzoy1}pyrrolidine-2-carboxaldehyde diethyl thioacetal VIII.

A solution of compound (2S) –N-{4-(5-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo[d]imidazol-2-yl] imidazol-2-y1]phenoxy)propoxy-5-methoxy-2-aminobenzoy1} pyrrolidine-2-carboxaldehyde diethyl thioacetal (765 mg, 1 mmol), $HgCl_2$ (613 mg, 2.26 mmol) and $CaCO_3$ (246 mg, 2.46 mmol) in MeCN-water (4:1) was stirred slowly at room temperature until TLC indicates completed loss of starting material. The reaction mixture was diluted with EtOAc (30mL) and filtered through a celite bed. The clear brown organic supernatant was extracted with saturated 5% $NaHCO_3$ (20mL), brine (20mL) and the combined organic phase was dried ($Na2SO_4$). The organic layer was evaporated in vacuum and purified by column chromatography (80% CH_2Cl_2-MeOH) to give compound 7-methoxy-8-(5-[4-[6-(4-methylhexahydro-1-pyraziny)-1H-benzo[d] imidazol-2-y1] phenoxy} butoxy)-(11aS)-1,2,3, - 11a-tetrahydro-5*H*-pyrrolo[2,1-c] [1,4] benzodiazepin-5-one.

^{1}H NMR ($CDCl_3$) & 1.75-2.95 (m,3H), 2.19-2.21 (m, 3H), 2.55-2.61 (m, 4H), 3.10-3.20 (m,4H), 3.60-3.80 (m, 3H), 3.85 (s,3H), 3.90-4.19 (m, 4H), 6.68 (s, 1H), 6.78-6.90 (m, 4H), 6.90 (s, 1H) 7.50-7.60 (d, 1H, J=4.4 Hz), 7.90-8.09 (d, 2H): MS (FAB) 623 $[M+H]^+$.

Example 7

Compound 4-[6-(4-methylhexahydro-1-pyrazonyl)-1*H*-zenzo [d] imidazol-2-yl] phenol X (342 g, 5 mmol) and (2S)-N-4-(3-bromobutyloxy)-5-methoxy-2-nitrobenzoy1] pyrrolidin-2-carboxaldehyde diethyl thioacetal of formula II (521 mg, 1 mmol) was taken in dry DMF (10mL). K_2CO_3 (690mg, 5 mmol) was added and the mixture was stirred for 12-24th. The reaction mixture was poured in to ice-water then solid was formed and it was filtered and aqueous media was extracted with EtOAc and $CHCl_3$ (50 mL), then the extracted solution was evaporated in vacuum to obtain the solid compound. Two solids were combined and the crude material was. Chromatographed over solica gel using chloroform / methanol (8:2) solvent to give compound (2S) –N-{3-(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo[d] imidazol-

2-yl] phenoxy) propoxy-5-method-2-nitrobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal VII as a sticky solid.

The compound (2S) –N-{3-(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo [d] imidazol-2-y1] phenoxy) propoxy-5-methoxy-2-nitrobenzoyl1} pyrrolidine-2-carboxaldehyde diethyl thioacetal XI (0.781 g, 1 mmol) was dissolved in methanol (15 ml) and added $SnCl_2.2H_2O$ (1.12 g, 5 mmol) was refluxed fro 2-5 h or until the TLC indicated that reaction was completed. The reaction mixture was then adjusted to pH 8 carefully with saturated $NaHCO_3$ solution, diluted with ethyl acetate, filtered through celite and extracted. The combined organic phase was dried over Na_2SO_4, and evaporated under vacuum to afford the crude compound (2S) –N-{3-(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo[d]imidazol-2-yl] imidazol-2-y1]phenoxy)propoxy-5-methoxy-2-aminobenzoy1}pyrrolidine-2-carboxaldehyde diethyl thioacetal XII.

A solution of compound (2S) –N-{3-(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo[d]imidazol-2-yl] imidazol-2-y1]phenoxy)propoxy-5-methoxy-2-aminobenzoy1} pyrrolidine-2-carboxaldehyde diethyl thioacetal (765 mg, 1 mmol), $HgCl_2$ (613 mg, 2.26 mmol) and $CaCO_3$ (246 mg, 2.46 mmol) in MeCN-water (4:1) was stirred slowly at room temperature until TLC indicates completed loss of starting material. The reaction mixture was diluted with EtOAc (30mL) and filtered through a celite bed. The clear brown organic supernatant was extracted with saturated 5% $NaHCO_3$ (20mL), brine (20mL) and the combined organic phase was dried (Na_2SO_4). The organic layer was evaporated in vacuum and purified by column chromatography (80% CH_2Cl_2-MeOH) to give compound 7-methoxy-8-(3-[4-[6-(4-methylhexahydro-1-pyraziny)-1*H*-benzo[d] imidazol-2-y1] phenoxy} butoxy)-(11aS)-1,2,3,-11a-tetrahydro-5*H*-pyrrolo[2,1-c] [1,4] benzodiazepin-5-one.

Example 8

Compound 4-[6-(4-methylhexahydro-1-pyrazonyl)-1*H*-zenzo [d] imidazol-2-yl] phenol X (342 g, 5 mmol) and (2S)-N-4-(4-bromobutyloxy)-5-methoxy-2-nitrobenzoy1] pyrrolidin-2-carboxaldehyde diethyl thioacetal of formula II (535 mg, 1 mmol) was taken in dry DMF (10mL). K_2CO_3 (690mg, 5 mmol) was added and the mixture was stirred for 12-24th. The reaction mixture was poured in to ice-water then solid was formed and it was filtered and aqueous media was extracted with EtOAc and $CHCl_3$ (50 mL), then the extracted solution was evaporated in vacuum to obtain the solid compound. Two solids were combined and the crude material was.

chromatographed over solica gel using chloroform / methanol (8:2) solvent to give compound (2S) –N-{4-(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo[*d*] imidazol-2-yl] phenoxy) butoxy-5-method-2-nitrobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal XI as a sticky solid.

The compound (2S) –N-{4(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo [d] imidazol-2-yl] phenoxy) propoxy-5-methoxy-2-nitrobenzoyll} pyrrolidine-2-carboxaldehyde diethyl thioacetal XI (0.795g, mmol) was dissolved in methanol (15 ml) and added $SnCl_2.2H_2O$ (1.12 g, 5 mmol) was refluxed fro 2-5 h or until the TLC indicated that reaction was completed. The reaction mixture was then adjusted to pH 8 carefully with saturated $NaHCO_3$ solution, diluted with ethyl acetate, filtered through celite and extracted. The combined organic phase was dried over Na_2SO_4, and evaporated under vacuum to afford the crude compound (2S) –N-{4-(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo[d]imidazol-2-yl] phenoxy) butoxy-5-methoxy-2-aminobenzoyl}pyrrolidine-2-carboxaldehyde diethyl thioacetal XII.

A solution of compound (2S) –N-{4-(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo[d]imidazol-2-yl] imidazol-2-yl]phenoxy)propoxy-5-methoxy-2-aminobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal (765 mg, 1 mmol), $HgCl_2$ (613 mg, 2.26 mmol) and $CaCO_3$ (246 mg, 2.46 mmol) in MeCN-water (4:1) was stirred slowly at room temperature until TLC indicates completed loss of starting material. The reaction mixture was diluted with EtOAc (30mL) and filtered through a celite bed. The clear brown organic supernatant was extracted with saturated 5% $NaHCO_3$ (20mL), brine (20mL) and the combined organic phase was dried (Na_2SO_4). The organic layer was evaporated in vacuum and purified by column chromatography (80% CH_2Cl_2-MeOH) to give compound 7-methoxy-8-(4-[4-[6-(4-methylhexahydro-1-pyraziny)-1*H*-benzo[d] imidazol-2-yl] phenoxy} butoxy)-(11aS)-1,2,3,-11a-tetrahydro-5*H*-pyrrolo[2,1-c] [1,4] benzodiazepin-5-one.

Example 9

Compound 4-[6-(4-methylhexahydro-1-pyrazonyl)-1*H*-zenzo [d] imidazol-2-yl] phenol X (342 g, 1 mmol) and (2S)-N-4-(5-bromobutyloxy)-5-methoxy-2-nitrobenzoyl] pyrrolidin-2-carboxaldehyde diethyl thioacetal of formula II (549 mg, 5 mmol) was taken in dry DMF (10mL). K_2CO_3 (690mg, 5 mmol) was added and the mixture was stirred for 12-24th. The reaction mixture was poured in to ice-water then solid was formed and it was filtered and aqueous media was extracted with EtOAc and $CHCl_3$ (50 mL), then the extracted solution was evaporated in vacuum to obtain the

solid compound. Two solids were combined and the crude material was chromatographed over silica gel using chloroform / methanol (8:2) solvent to give compound (2S) –N-{5-(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo[*d*] imidazol-2-yl] phenoxy) pentyloxy-5-method-2-nitrobenzoyl} pyrrolidine-2-carboxaldehyde diethyl thioacetal XI as a sticky solid.

The compound (2S) –N-{5(4-[6-(4-methylhexahydro-1-pyraziny1)-1*H*-benzo [d] imidazol-2-y1] phenoxy) pentyloxy -5-methoxy-2-nitrobenzoyl1} pyrrolidine-2-carboxaldehyde diethyl thioacetal XI (0.809 g, 1 mmol) was dissolved in methanol (15 ml) and added $SnCl_2.2H_2O$ (1.12 g, 5 mmol) was refluxed fro 2-5 h or until the TLC indicated that reaction was completed. The reaction mixture was then adjusted to pH 8 carefully with saturated $NaHCO_3$ solution, diluted with ethyl acetate, filtered through celite and extracted. The combined organic phase was dried over Na_2SO_4, and evaporated under vacuum to afford the crude compound (2S) –N-{5-(4-[6-(4-etyylhexahydro-1-pyraziny1)-1*H*-benzo[d]imidazol-2-yl] phenoxy) pentyloxy-5-methoxy-2-aminobenzoyl} pyrrolidine –2-carboxaldehyde diethyl thioacetal XII.

A solution of compound (2S) –N-{5-(4-[6-(4-ethylhexahydro-1-pyraziny1)-1*H*-benzo[d]imidazol-2-yl] phenoxy)pentyloxy-5-methoxy-2-aminobenzoy1} pyrrolidine-2-carboxaldehyde diethyl thioacetal XII (779 mg, 1 mmol), $HgCl_2$ (613 mg, 2.26 mmol) and $CaCO_3$ (246 mg, 2.46 mmol) in MeCN-water (4:1) was stirred slowly at room temperature until TLC indicates completed loss of starting material. The reaction mixture was diluted with EtOAc (30mL) and filtered through a celite bed. The clear brown organic supernatant was extracted with saturated 5% $NaHCO_3$ (20mL), brine (20mL) and the combined organic phase was dried (Na_2SO_4). The organic layer was evaporated in vacuum and purified by column chromatography (80% CH_2Cl_2-MeOH) to give compound 7-methoxy-8-(5-[4-[6-(4-ethylhexahydro-1-pyraziny)-1*H*-benzo[d] imidazol-2-y1] phenoxy} pentyloxy)-(11aS)-1,2,3,-11a-tetrahydro-5*H*-pyrrolo[2,1-c] [1,4] benzodiazepin-5-one.

Biological activity: in vitro biological activity studies were carried out at National Cancer Institute (USA).

Cytotoxicity: Compound IX was evaluated for the primary anti-cancer activity (Table-1) and *in vitro* against sixty human tumour cells derived from nine cancer types (leukemia, non-small-cell lung, colon, CNS, melanoma, ovarian, prostate, and breast cancer). For each compound, dose response curves for each cell line were measured at a minimum of five concentrations at 10 fold dilutions. A protocol of 48 h continuous

drug exposure was used and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. The concentration causing 50% cell growth inhibition (GI50), total cell growth control was calculated. The mean graph midpoint values of log10 TGI and log10 LC50 as well as log 10 GI50 for VI are listed in Table 2. As demonstrated by mean graph pattern, compound IV exhibits an interesting profile of activity and selectivity for various cell lines. The mean graph mid point of log10 TGI and log10 LC50 showed similar pattern to the log10 GI 50 mean graph mid points.

Table 1: in vitro one dose primary anticancer assay[a] of PBD hybrid formula IX as representative compound.

PBD hybrid	**Growth Percentages.**		
	(Lung) NCI-H460	(Breast) MCF7	(CNS) SF-268
IX	0	0	0

[A]One dose of IX at 10^{-4} molar concentration.

The novel pyrrolobenzodiazepine hybrid of formula IX has shown to possess 10 nano molar potency (at the LC50 level) against one non-small cell lung caner (NCI-H522), one CNS cancer (SF-539), three melanoma cancer (SK-MEL-2-SK-MEL-5,VACC-62), two renal cancer (A-498, RXF 393), and one breast cancer (MDA-MB-435). The LC 50 values of nine cancer (average of six to nine cancer cell line) of compound IX listed in **Table 3.**

Table 2. log10 GI50 log10 TGI and LC50 mean graphs midpoints (MG_MID) of in vitro cytotoxicity data for the compound IX as representative compound against human tumour cell lines.

Compound	Log10 GI50	Log10 TGI	Log10LC50
V	-7.97	-6.79	-4.57

437-NF-03

Table 3. Log LC50 (concentration in mol/L causing 50% lethality) Values for Compounds IX as representative compound.

Cancer	Compound
Leukemia	>4.0
Non-small-cell lung	-4.89
Colon	>-4.03
CNS	-4.12
Melanoma	-5.84
Ovarian	>-4.23
Renal	-4.33
Prostate	-4.48
Breast	>-4.65

Each cancer type represents the average of six to nine different cancer cell lines.

Table 4- MEAN GRAPH